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Biological Control of *Cucurbita pepo* var. *texana* (Texas Gourd) in Cotton (*Gossypium hirsutum*) with the Fungus *Fusarium solani* f. sp. *cucurbitae*

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ABSTRACT

Experiments were conducted to evaluate various formulations and application methods of the fungus *Fusarium solani* f. sp. *cucurbitae* (FSC) for controlling Texas gourd (*Cucurbita pepo* var. *texana*) in cotton. In greenhouse tests, Texas gourd was controlled 93% and 96%, respectively, with pre-emergence applications of FSC-infested cornmeal/sand medium (CMS) and FSC-wheat flour/kaolin ('Pesta') granules. Post-emergence applications of CMS or 'Pesta' granular formulations were less effective overall. However, >90% control of Texas gourd was achieved with post-emergence applications of FSC spores formulated in an emulsion consisting of 25% unrefined corn oil and 0.2% Silwet L-77 surfactant. Dew was not required to achieve optimal levels of weed control with either the pre-emergence granular formulations or with post-emergence corn oil/surfactant applications. In field tests, pre-emergence applications of FSC-infested CMS and FSC-'Pesta' granules controlled 90-94% of the weeds. Post-emergence applications of FSC formulated in corn oil/surfactant were equally efficacious in controlling Texas gourd in cotton. No damage to cotton was observed.

Keywords: bioherbicide, *Cucurbita pepo* var. *texana*, *Fusarium solani* f. sp. *cucurbitae*, mycoherbicide, oil-in-water emulsion, surfactant, unrefined corn oil

Abbreviations: CO, unrefined corn oil; **CMS**, cornmeal/sand medium; **FSC**, *Fusarium solani* f. sp. *cucurbitae*; **POE**, post-emergence; **PE**, pre-emergence; **SW**, Silwet L-77 surfactant

INTRODUCTION

Cucurbita pepo var. *texana* (Scheele) D. Decker (Cucurbitaceae; Texas gourd) is an escaped ornamental (Erwin 1938) that has become a weed problem in localized areas of the Arkansas and Red River Valleys of Arkansas (Lee and Oliver 1982; Oliver *et al*. 1983). Texas gourd has been a relatively minor weed in cotton and soybean in Mississippi for several decades, but it has become increasingly more common and problematic during recent years (Bryson and Byrd 1996). Its distribution increased, so that over the period from 1991-1998, it had spread into 12 counties. Specimens and/or photographs of this weed from many sites have been entered in the SWSL Herbarium at Stoneville, Mississippi, USA. This weedy vine produces fruit (pepos) containing abundant seeds. After the pepos mature and dry, they can be spread and dispersed mechanically, e.g., during cultivation, or the dry pepos can be transported via floatation in ditches and waterways. Thus it is highly probable that Texas gourd has spread further in Mississippi, especially in areas of the Mississippi River Alluvial Plain Region. This weed competes for water and nutrients and interferes with harvesting, and the weed problem is perpetuated if infested fields are abandoned (Oliver *et al*. 1983).

Texas gourd can be controlled in soybean by some pre- and post-emergence herbicides, although effective control depends upon timing, and soil and climatic conditions (Oliver *et al*. 1983). Effective chemical control in soybean can be complicated by the need for repeated applications due to continual emergence of Texas gourd under favorable conditions throughout the growing season (Weidemann and Templeton 1988). Oliver *et al*. (1983) demonstrated that no single herbicide or herbicide mix provided a consistent level of Texas gourd control over all years and locations that were tested in Arkansas. Consistent control is difficult since pre-emergence herbicides such as metribuzin are rainfall-dependent and post-emergence herbicides must be applied by the V2 stage of soybean growth and repeated at the V4 stage or as needed (Oliver *et al*. 1983).

The herbicide mixes used for Texas gourd control in soybean are not applicable for use in cotton because of crop injury. Continuous seed germination and the ability to root at the nodes minimize the efficacy of mechanical control. The weed is particularly troublesome in cotton because many chemical herbicides such as cyanazine [2-(4-chloro-6-ethylamino-1, 3, 5-triazin-2-ylamino)-2-methylpropionitrile] and dinoseb [2-(sec-butyl)-4,6-dinitrophenol] that formerly provided excellent control of this weed are no longer available (Bryson and Byrd 1996). It has been also shown that Texas gourd is tolerant to the herbicide clomazone [2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone] and that widespread use of this herbicide alone seemed to favor increased Texas gourd populations (Smeda 1997). Clearly, alternative control measures are needed for this onerous weed.

The fungus *Fusarium solani* App. & Wr. f. sp. *cucurbitae* Snyd. & Hans (FSC) [ATTC repository no. 52552], originally isolated from greenhouse-grown Texas gourd seedlings, was shown to incite a severe collar rotting disease on seedlings and mature Texas gourd plants (Boyette *et al*. 1984) The pathogen was evaluated as a mycoherbicide in Arkansas at several locations over diverse environmental and climatic conditions, and provided excellent Texas gourd control in soybean (Boyette *et al*. 1984; Weidemann and Templeton 1988; Weidemann *et al*. 1988), but FSC was not evaluated for controlling this weed in cotton. Because U.S. cotton production utilizes several cultural practices that can be potentially detrimental to the efficacy of candidate bioherbicides, such as in-furrow fungicides and insecticides (Boyette and Bryson 1998), the utility of FSC as a bioherbi-

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cide in cotton was uncertain. The present study was therefore undertaken to evaluate the bioherbicidal efficacy of various solid and liquid formulations of FSC and application methods under controlled environments, and to evaluate these formulations for controlling Texas gourd in cotton field trials.

MATERIALS AND METHODS

Inoculum and formulation production

Inoculum for liquid formulations was produced on modified Richard's medium containing vegetable juice (V-8 vegetable juice, Campbell Soup Co., Camden, NJ, USA) (Daniel *et al.* 1973) contained in shake flasks (125 rpm) at 28°C. A stable oil-in-water emulsion formulation was prepared by adding the aqueous fungal component to 25% (v/v) unrefined corn oil (CO) [Spectrum Naturals, Petaluma, CA, USA] and 0.2% (v/v) Silwet L-77 surfactant (SW) [OSi Specialties, Inc., Charlotte, NC, USA] (Boyette 1994). Granular formulations consisted of FSC-infested cornmeal/sand (5% w/w) (CMS) (Boyette *et al.* 1984) and FSC-infested wheat gluten flour granules ('Pesta') prepared as described by Connick *et al*. (1991). Briefly, the aqueous fungal homogenate (23 ml) was added to an 80:20 (w/w) mixture of semolina (durum) wheat flour (Tropical Nut & Fruit Co., Charlotte, NC, USA), and kaolin (Thiele Kaolin Co., Wren, GA, USA). The mixture was kneaded to form dough, pressed flat, folded by hand five times, and extruded through a table-mounted pasta maker (Atlas Model 150, Tantonio Co., Eastlake, OH, USA). The sheets (1.0-1.5 mm thick) were air-dried on elevated polyester screens for 3 days at 22°C and 50-60% relative humidity. The dried sheets were broken, milled, and sieved to obtain #14- to 18-mesh (1-2 mm) granules, and stored at 4°C. Inoculum concentrations were adjusted so as to provide a final concentration of 2.0×10^7 spores g^{-1} (solid) or ml^{-1} (liquid) of carrier for all formulations. For all greenhouse and field experiments, application rates were 300 L ha^{-1} for liquid formulations and 100 kg ha^{-1} for granular formulations.

Greenhouse experiments

Texas gourd seeds (collected near Leland, MS, USA) were surface-sterilized in 0.05% NaOCl for 5 min, rinsed with sterile distilled water, and germinated on moistened filter paper in Petri dishes. After the seeds germinated (~ 48 h) they were planted in a commercial potting mix (Jiffy-mix; Jiffy Products of America, Batavia, IL, USA) contained in peat strips. Each strip contained 12 plants, and three strips were used for each treatment, for a total of 36 plants for each treatment. The potting mix was supplemented with a controlled-release (14: 14: 14, NPK) fertilizer (Osmocote; Grace Sierra Horticultural Products, Milpitas, CA, USA). The plants were placed in subirrigated trays that were mounted on greenhouse benches. Greenhouse temperatures ranged from 25 to 30°C with 40-90% relative humidity (RH). The photoperiod was approximately 14 h, with 1800 PAR as measured at midday with a light meter. Treatments consisted of pre-emergence or post-emergence applications of: (1) FSC/water; (2) FSC/CO/SW (3) FSC/CMS; (4) FSC/'Pesta'; (5) CO/SW control; (6) inert CMS; (7) inert 'Pesta'; and (8) untreated control. For plants receiving pre- or post-emergence treatments, inoculum was applied either with hand-held aerosol sprayers (Spra-Tool, AERVOE Industries, Gardnerville, NV, USA) or by sprinkling granular inoculum on soil surfaces at rates equivalent to those in field tests (*ca*. 100 kg ha^{-1}). For plants receiving a dew treatment, the inoculated plants were placed in darkened dew chambers (Model I-35 DL, Percival Scientific, Perry, IA, USA) at 25°C and 100% relative humidity (RH) for 12 h. Following the dew treatment, plants were placed in subirrigated trays that were mounted on greenhouse benches and monitored for disease development and mortality. Mortality was determined 14 days after inoculation. Greenhouse temperatures ranged from 25 to 30°C with 40-90% RH. The photoperiod was 12 h with 1,650 µmol m^{-2} s^{-1} photosynthetically active radiation measured at midday. All greenhouse experiments were repeated in time and data were pooled after subjecting to homogeneity of variance (Steele *et al*. 1997).

Field experiments

Field experiments were conducted on a Dundee very fine sandy loam (Aeric Ochraqualf) in 1997-1998 near Stoneville, MS, USA (33° 26′ N, 90° 53′ W, as determined using a global positioning device, Garmin, Model Oregon 300, Garmin Ltd., Olathe, KS, USA) that was naturally infested with Texas gourd. Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) was applied pre-plant incorporated (PPI) to the test area at a broadcast rate of 1.12 kg ha^{-1} for grass control. Standard cotton production practices were utilized: acephate (O,S-dimethyl acetylphos-phoramidothioate), and carboxin (5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carbamil), for systemic control of several fungal pathogens, PCNB (pentachloronitrobenzene) for control of damping–off disease and the systemic insecticide disulfoton (O,O-diethyl S-[2-(ethylthio)ethyl]phosphorodithioate). Test plots consisted of four rows of cotton (cv 'Suregrow', Delta Pine Inc., Rosedale, MS, USA) 6.1 m long and 1 m apart with the two center rows receiving treatment. Additional Texas gourd seeds were planted to ensure a uniform stand of 25 seed m^{-1} of row. Planting dates were June 12, 1997 and June 5, 1998. Treatments were as used above in the greenhouse tests: (1) FSC/water; (2) FSC/CO/SW (3) FSC/CMS; (4) FSC/'Pesta'; (5) CO/SW control; (6) inert CMS; (7) inert 'Pesta'; and (8) untreated control. Each treatment was applied pre-emergence or post-emergence. In plots receiving pre-emergence applications, the treatments were made at the time of planting. Pre-emergence CMS and 'Pesta' applications were applied by hand in a 20 cm band to planted cotton seedlings (20-25 cm tall). Pre-emergence liquid formulations were applied as a band as described above using a back-pack sprayer (Spray doc, Model 101P; Gilmour Mfg., Somerset, PA, USA). Liquid formulation applications in post-emergence treatments were applied manually with back-pack sprayers at a spray rate of 300 L ha^{-1} when Texas gourd seedlings were in the cotyledonary-to-first leaf growth stage. All post-emergence CMS and 'Pesta' applications were applied to the soil surface in 20-cm bands to the treated rows at a rate of 100 kg ha^{-1}. Percentages of weed control and disease progression of Texas gourd were determined in randomly selected 3.0- by 0.46-m areas at 3, 7, 14, 21, and 28 days after inoculation. The extent of disease progression was based on a modified Horsfall-Barratt (1945) rating scale, assigning symptom expression from 0 to 1.0, with 0 being unaffected, and 0.2, 0.4, 0.6, 0.8 = 20%, 40%, 60%, and 80% leaf and stem wilt injury, respectively, and 1.0 = plant mortality. Symptomatology was considered "severe" at ratings of 0.8 to 1.0.

Statistical analysis

The experiments were arranged as randomized complete block designs with four replications. Data over the two year field testing period were examined for homogeneity of variance (Steele *et al*. 1997), combined, and analyzed using anova. Non-transformed data are presented because arcsine square-root transformation of data did not alter interpretation of data. When significant differences were detected by the *F*-test, means were separated with Fisher's protected LSD test at the 0.05 level of probability. In the disease kinetic studies, data were analyzed using SEM and linear regression analysis.

RESULTS AND DISCUSSION

Greenhouse experiments

Texas gourd was controlled 93-96% with pre-emergence applications of FSC-infested CMS and with FSC-'Pesta' granules (**Fig. 1**). Post-emergence applications of granular formulations were less effective overall, but over 90% control was achieved with post-emergence applications of the FSC-corn oil/surfactant formulation (**Fig. 1**). Dew was not required to effectively control Texas gourd with either the pre-emergence granular formulations or with post-emergence corn oil/surfactant formulations (**Fig. 1**).

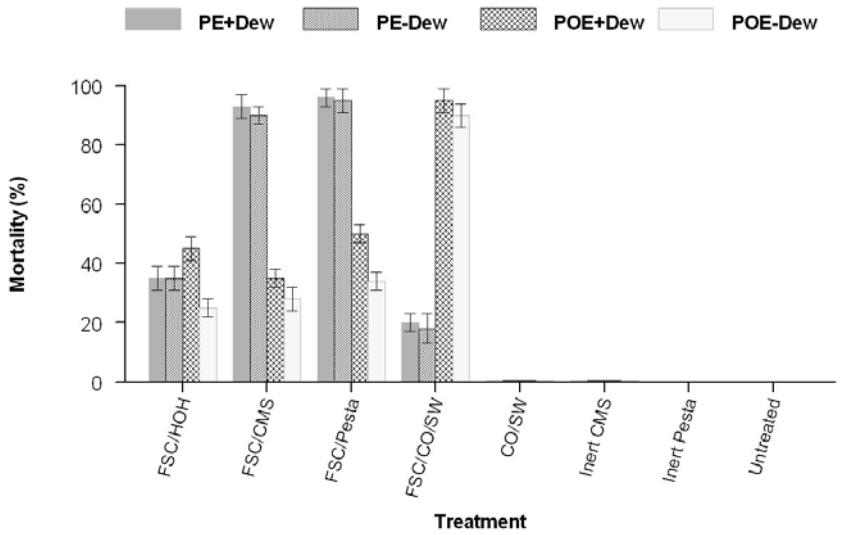

Fig. 1 Effect of formulation on biocontrol of Texas gourd treated pre-emergence (PE) and post-emergence (POE) in greenhouse experiments with *Fusarium solani* f. sp. *Cucurbitae*, with and without a 12 h dew treatment. Error bars represent significant differences at P = 0.05 according to Fisher's protected LSD.

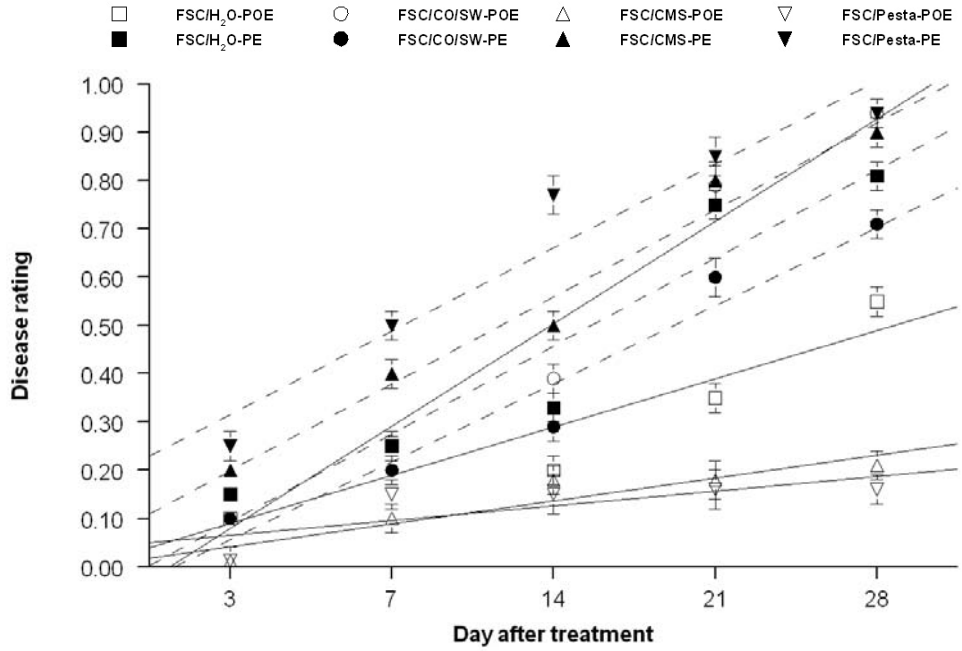

Fig. 2 Disease progression of *Fusarium solani* f. sp *cucurbitae* (FSC)-infected Texas gourd resulting from pre-emergence (PE) or post-emergence (POE) field applications over a period of 28 days after treatment. Proportion of diseased plants was based on symptom expression from 0 to 1.0, with 0 being unaffected and 1.0 plant mortality. Symptomatology was considered "severe" at ratings of 0.8 to 0.10. In PE treatments, for plants treated FSC/H_2O, the relationship is best described by the equation: Y = + 0.518 – 0.598 X, R^2 = 0.96. For plants treated with FSC/CO/SW, the relationship is best described by the equation: Y = + 0.28 – 0.0.314 X, R^2 = 0.98. For plants treated with FSC/CMS, the relationship is best described by the equation: Y = + 0.16 – 0.017 X, R^2 = 0.97. For plants treated with FSC/'Pesta', the relationship is best described by the equation Y = + 0.098 – 0.372 X, R^2 = 0.98. In POE treatments, for plants treated with FSC/H_2O, the relationship is best described by the equation: Y = -0.21 + 0.463 X, R^2 = 0.96. For plants treated with FSC/CO/SW, the relationship is best described by the equation: Y = + 0.454 – 0.513 X, R^2 = 0.98. For plants treated with FSC/CMS, the relationship is best described by the equation: Y = - 0.158 + 0.206 X, R^2 = 0.98. For plants treated with FSC/'Pesta', the relationship is best described by the equation: Y = - 0.276 + 0.394 X, R^2 = 0.96. Error bars represent ± 1 S.E.M.

Field experiments

Results from the field experiments corroborated our findings in the greenhouse. Pre-emergence applications of FSC/CMS and FSC/'Pesta' granules controlled (killed) 90 and 94% of Texas gourd plants, respectively (**Table 1**). Post-emergence applications of FSC formulated in corn oil/surfactant were equally efficacious in controlling Texas gourd (**Table 1**). Texas gourd plants treated postemergence with FSC/CO/SW exhibited a severe collar-rot that eventually girdled the stem resulting in wilting and mortality (**Fig. 2**). Pre-emergence applications of FSC/water or corn oil/Silwet L-77 were less effective overall, although 81 and 71% control of Texas gourd was achieved in FSC/water, and FSC/CO/SW plots, respectively (**Table 1**). Post-emergence applications of FSC/water provided only 55% control, whereas, FSC/CO/SW provided 94% control (**Table 1**). In pre-emergence applications, the FSC/'Pesta' treatment caused the most rapid promotion of disease, and FSC/CO/SW was the least effective in promoting disease symptommology. Applications of FSC/H_2O and FSC/CMS caused intermediate disease progression (**Fig. 2**). In test plots receiving post-emergence applications, disease progression was generally slower, with greatly reduced infection levels of all treatments except plots treated with FSC/CO/SW. FSC/CO/SW caused a very rapid increase in disease prog-

Table 1 Biological control of Texas gourd with *Fusarium solani* f. sp. *cucurbitae* in cotton field test plots[a-c].

Application and inoculum type	Texas gourd control (%)
Pre-emergence	
FSC/H_2O	81 b
FSC/CO/SW	71 c
FSC/CMS	90 a
FSC/'Pesta'	94 a
CO/SW control	11 fg
H_2O control	6 g
Inert CMS	4 g
Inert 'Pesta'	6 g
Untreated	4 g
Post-emergence	
FSC/H_2O	55 d
FSC/CO/SW	94 a
FSC/CMS	21 e
FSC/'Pesta'	16 ef
CO/SW control	14 f
H_2O control	6 g
Inert CMS	2 g
Inert "Pesta"	4 g
Untreated	0 g

[a] Abbreviations: CO, unrefined corn oil; CMS, cornmeal-sand medium FSC, *Fusarium solani f. sp. cucurbitae*; SW, Silwet L-77 surfactant.
[b] Inoculum concentrations were adjusted to 2×10^7 spores/g carrier for all formulations. Application rates were 300 L ha^{-1} for liquid formulations and 100 kg ha^{-1} for granular formulations.
[c] Means followed by the same letter are not significantly different according to Fisher's protected LSD at P=0.05.

ression from 14 to 28 DAT (**Fig. 2**). No visible damage occurred to cotton with any of the treatments (data not shown).

These results suggest that some formulations *Fusarium solani* f. sp. *cucurbitae*, applied appropriately can be effective in controlling Texas gourd in cotton. Satisfactory post-emergence and pre-emergence control can be achieved using either granular or liquid formulations. Because the test was not taken to yield, we were unable to follow the fate of this pathogen in cotton test plots or its ability to control late-emerging weeds during the entire growing season. Previous research has shown that FSC can persist at sufficient levels to control Texas gourd for at least 3 months in either soybean or fallowed plots (Boyette *et al*. 1984). The research presented here has shown that FSC can persist at levels sufficient to control weeds for at least 4 weeks in all FSC formulations applied pre-emergence, but only FSC/CO/SW applied post-emergence provided substantial weed control after 4 weeks (**Table 1**). Previous research has shown that certain cotton pesticides, such as acephate, carboxin, and PCNB can adversely affect the bioherbicidal efficacy of the anthracnose-forming pathogen *Colletotrichum truncatum* (Schw.) Andrus & Moore for controlling *Sesbania exaltata* (Raf.) Rydb. ex Cory. (Fabaceae; hemp sesbania) post-emergence in cotton (Boyette and Bryson 1998). However, we did not observe these interactions in the present research with FSC when these compounds were used in a routine spray program for cotton in our test plots. It has been shown that some herbicides promote disease development of various plant pathogens, especially *Fusarium* spp. For example, Yu *et al*. (1988) demonstrated that growth and respiration of FSC *in vivo* is stimulated by trifluralin. Weidemann and Templeton (1988) further demonstrated that pre-emergence applications of FSC tank-mixed with trifluralin, significantly reduced weed emergence and increased mortality of Texas gourd above levels obtained with either the fungus or herbicide alone (Weidemann and Templeton 1988).

FSC produces three types of spores: microconidia, macroconidia, and chlamydospores. All spore types are equivalent with regard to pathogenicity, but have variable persistence in nature, with microconidia being the least persistent and chlamydospores the most persistent (Nash and Alexander 1965; Boyette *et al.* 1984). In the research presented here, roughly equivalent mixtures of each spore type were used (data not shown). However, it is possible to obtain almost pure quantities of each spore type by modifying the nutritional amendments and inoculum production methods (Boyette *et al.* 1984; Weidemann *et al.* 1988). This would make it possible to "customize" production of FSC inoculum for use either in pre-emergence or post-emergence applications, as required.

Recent research has suggested that hybridization between genetically modified crops and their weedy or wild relatives could possibly result in "superweeds" that are resistant to various chemical herbicides (Anonymous 1996). Spencer and Snow (2001) compared fitness components of Texas gourd collected in Arkansas, USA with wild-crop hybrids derived from yellow squash (*C. pepo* L.) that had transgenic resistance to two viruses and found that virus symptoms were more common in wild plants than in hybrids, suggesting that crop genes may be passed into free-living populations of Texas gourd. It is therefore possible that this weed may become more prominent and provide a niche market for a biological control agent, such as *F. solani* f. sp. *cucurbitae*.

If pursued, future research should include testing the effects of currently used cotton pesticides on the growth and biocontrol efficacy of FSC. Since the range of Texas gourd appears to be increasing, and chemicals that can control the weed are being removed from the market, there may be a greater potential to utilize this pathogen as a bioherbicide for controlling this troublesome weed.

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